

Tany



PCT10

## RAW SEQUENCE LISTING

DATE: 06/12/2002

PATENT APPLICATION: US/10/049,358

TIME: 12:22:42

Input Set : A:\10-049,358-Sequence Listing.txt

Output Set: N:\CRF3\06122002\J049358.raw

3 <110> APPLICANT: Davis, Maria  
 4 Nelson, John  
 5 Kumar, Shiv  
 6 Finn, Patrick  
 7 Nampalli, Satyam  
 8 Flick, Parke  
 10 <120> TITLE OF INVENTION: TAQ DNA Polymerase Having an Amino Acid Substitution at  
 11 E681 and Homologs Thereof Exhibiting Improved Salt  
 12 Tolerance  
 14 <130> FILE REFERENCE: PB9944  
 16 <140> CURRENT APPLICATION NUMBER: 10/049,358  
 C--> 17 <141> CURRENT FILING DATE: 2002-05-17  
 19 <150> PRIOR APPLICATION NUMBER: PCT/US00/22150  
 20 <151> PRIOR FILING DATE: 2000-08-10  
 22 <150> PRIOR APPLICATION NUMBER: 60/148,012  
 23 <151> PRIOR FILING DATE: 1999-08-10  
 25 <160> NUMBER OF SEQ ID NOS: 3  
 27 <170> SOFTWARE: PatentIn Ver. 2.1  
 29 <210> SEQ ID NO: 1  
 30 <211> LENGTH: 832  
 31 <212> TYPE: PRT  
 32 <213> ORGANISM: Thermus aquaticus  
 34 <400> SEQUENCE: 1  
 35 Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu  
 36 1 5 10 15  
 38 Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly  
 39 20 25 30  
 41 Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala  
 42 35 40 45  
 44 Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val  
 45 50 55 60  
 47 Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly  
 48 65 70 75 80  
 50 Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu  
 51 85 90 95  
 53 Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu  
 54 100 105 110  
 56 Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys  
 57 115 120 125  
 59 Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp  
 60 130 135 140  
 62 Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly  
 63 145 150 155 160

ENTERED

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65 Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
66                               165                               170                               175
68 Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
69                               180                               185                               190
71 Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
72                               195                               200                               205
74 Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
75                               210                               215                               220
77 Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
78 225                               230                               235                               240
80 Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
81                               245                               250                               255
83 Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe
84                               260                               265                               270
86 Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu
87                               275                               280                               285
89 Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly
90                               290                               295                               300
92 Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp
93 305                               310                               315                               320
95 Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro
96                               325                               330                               335
98 Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu
99                               340                               345                               350
101 Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro
102                               355                               360                               365
104 Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn
105                               370                               375                               380
107 Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu
108 385                               390                               395                               400
110 Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu
111                               405                               410                               415
113 Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu
114                               420                               425                               430
116 Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly
117                               435                               440                               445
119 Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala
120                               450                               455                               460
122 Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His
123 465                               470                               475                               480
125 Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp
126                               485                               490                               495
128 Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg
129                               500                               505                               510
131 Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile
132                               515                               520                               525
134 Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr
135                               530                               535                               540
137 Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu

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138 545      550      555      560
140 His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
141      565      570      575
143 Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
144      580      585      590
146 Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
147      595      600      605
149 Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
150      610      615      620
152 Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
153 625      630      635      640
155 Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
156      645      650      655
158 Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
159      660      665      670
161 Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
162      675      680      685
164 Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
165      690      695      700
167 Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
168 705      710      715      720
170 Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
171      725      730      735
173 Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
174      740      745      750
176 Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
177      755      760      765
179 Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
180      770      775      780
182 Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
183 785      790      795      800
185 Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
186      805      810      815
188 Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
189      820      825      830
195 <210> SEQ ID NO: 2
196 <211> LENGTH: 560
197 <212> TYPE: PRT
198 <213> ORGANISM: Thermus aquaticus
200 <400> SEQUENCE: 2
201 Met Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu
202 1      5      10      15
204 Leu Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu
205      20      25      30
207 Gly Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala
208      35      40      45
210 Asp Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala
211      50      55      60
213 Pro Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu

```

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214	65					70				75					80	
216	Leu	Ala	Lys	Asp	Leu	Ser	Val	Leu	Ala	Leu	Arg	Glu	Gly	Leu	Gly	Leu
217					85					90					95	
219	Pro	Pro	Gly	Asp	Asp	Pro	Met	Leu	Leu	Ala	Tyr	Leu	Leu	Asp	Pro	Ser
220				100					105					110		
222	Asn	Thr	Thr	Pro	Glu	Gly	Val	Ala	Arg	Arg	Tyr	Gly	Gly	Glu	Trp	Thr
223			115					120					125			
225	Glu	Glu	Ala	Gly	Glu	Arg	Ala	Ala	Leu	Ser	Glu	Arg	Leu	Phe	Ala	Asn
226		130					135					140				
228	Leu	Trp	Gly	Arg	Leu	Glu	Gly	Glu	Glu	Arg	Leu	Leu	Trp	Leu	Tyr	Arg
229	145				150					155					160	
231	Glu	Val	Glu	Arg	Pro	Leu	Ser	Ala	Val	Leu	Ala	His	Met	Glu	Ala	Thr
232				165					170					175		
234	Gly	Val	Arg	Leu	Asp	Val	Ala	Tyr	Leu	Arg	Ala	Leu	Ser	Leu	Glu	Val
235			180						185					190		
237	Ala	Glu	Glu	Ile	Ala	Arg	Leu	Glu	Ala	Glu	Val	Phe	Arg	Leu	Ala	Gly
238			195				200					205				
240	His	Pro	Phe	Asn	Leu	Asn	Ser	Arg	Asp	Gln	Leu	Glu	Arg	Val	Leu	Phe
241		210				215					220					
243	Asp	Glu	Leu	Gly	Leu	Pro	Ala	Ile	Gly	Lys	Thr	Glu	Lys	Thr	Gly	Lys
244	225				230					235					240	
246	Arg	Ser	Thr	Ser	Ala	Ala	Val	Leu	Glu	Ala	Leu	Arg	Glu	Ala	His	Pro
247				245					250					255		
249	Ile	Val	Glu	Lys	Ile	Leu	Gln	Tyr	Arg	Glu	Leu	Thr	Lys	Leu	Lys	Ser
250			260					265					270			
252	Thr	Tyr	Ile	Asp	Pro	Leu	Pro	Asp	Leu	Ile	His	Pro	Arg	Thr	Gly	Arg
253			275				280					285				
255	Leu	His	Thr	Arg	Phe	Asn	Gln	Thr	Ala	Thr	Ala	Thr	Gly	Arg	Leu	Ser
256		290				295					300					
258	Ser	Ser	Asp	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Thr	Pro	Leu	Gly
259	305				310					315					320	
261	Gln	Arg	Ile	Arg	Arg	Ala	Phe	Ile	Ala	Glu	Gly	Trp	Leu	Leu	Val	
262				325					330				335			
264	Ala	Leu	Asp	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Leu	Ser
265			340				345					350				
267	Gly	Asp	Glu	Asn	Leu	Ile	Arg	Val	Phe	Gln	Glu	Gly	Arg	Asp	Ile	His
268			355				360				365					
270	Thr	Glu	Thr	Ala	Ser	Trp	Met	Phe	Gly	Val	Pro	Arg	Glu	Ala	Val	Asp
271		370				375					380					
273	Pro	Leu	Met	Arg	Arg	Ala	Ala	Lys	Thr	Ile	Asn	Tyr	Gly	Val	Leu	Tyr
274	385				390					395					400	
276	Gly	Met	Ser	Ala	His	Arg	Leu	Ser	Gln	Arg	Leu	Ala	Ile	Pro	Tyr	Glu
277				405					410				415			
279	Glu	Ala	Gln	Ala	Phe	Ile	Glu	Arg	Tyr	Phe	Gln	Ser	Phe	Pro	Lys	Val
280			420				425				430					
282	Arg	Ala	Trp	Ile	Glu	Lys	Thr	Leu	Glu	Glu	Gly	Arg	Arg	Arg	Gly	Tyr
283			435				440				445					
285	Val	Glu	Thr	Leu	Phe	Gly	Arg	Arg	Arg	Tyr	Val	Pro	Asp	Leu	Glu	Ala
286		450				455				460						

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288 Arg Val Lys Ser Val Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
289 465                               470                               475                               480
291 Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
292                               485                               490                               495
294 Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
295                               500                               505                               510
297 Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
298                               515                               520                               525
300 Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
301                               530                               535                               540
303 Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
304 545                               550                               555                               560
310 <210> SEQ ID NO: 3
311 <211> LENGTH: 830
312 <212> TYPE: PRT
313 <213> ORGANISM: Thermus aquaticus
315 <400> SEQUENCE: 3
316 Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
317 1                               5                               10                               15
319 Val Ala Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly
320                               20                               25                               30
322 Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
323                               35                               40                               45
325 Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
326                               50                               55                               60
328 Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
329 65                               70                               75                               80
331 Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu
332                               85                               90                               95
334 Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu
335                               100                              105                              110
337 Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys
338                               115                              120                              125
340 Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp
341                               130                              135                              140
343 Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly
344 145                               150                              155                              160
346 Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro
347                               165                              170                              175
349 Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn
350                               180                              185                              190
352 Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu
353                               195                              200                              205
355 Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu
356                               210                              215                              220
358 Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys
359 225                               230                              235                              240
361 Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val
362                               245                              250                              255

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**VERIFICATION SUMMARY**

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Input Set : A:\10-049,358-Sequence Listing.txt

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L:17 M:271 C: Current Filing Date differs, Replaced Current Filing Date